UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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FEB 23 1983

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

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SUBJECT: PP#2F2707/FAP#2H5355 Cyromazine use in Poultry Feed. Review of Analytical Method, and Metabolism and Feeding Studies.

FROM: Philip V. Errico, Chemist
Residue Chemistry Branch
Hazard Evaluation Division (TS-769)

THRU: Charles L. Trichilo, Chief
Residue Chemistry Branch
Hazard Evaluation Division (TS-769)

TO: Franklin Gee, PM#17
Insecticide-Rodenticide Branch
Registration Division (TS-767)

and

Toxicology Branch
Hazard Evaluation Division (TS-769)

Ciba-Geigy has submitted a request for permanent tolerances of 0.4 ppm for cyromazine (N-cyclopropyl-1,3,5-triazine-2,4,6-triamine, CGA-72662) and its metabolite, melamine (1,3,5-triazine-2,4,6-triamine) in or on eggs, meat, fat, and meat by-products of poultry.

A feed additive tolerance of 5.0 ppm is proposed for residues of cyromazine in poultry feed.

These tolerances are proposed for the use of cyromazine in poultry feed to control flies in poultry manure.

We have recommended for the petitioner's requested temporary tolerances of 0.2 ppm in/on eggs, meat, fat and meat by-products of poultry, 0.1 ppm in/on meat, fat, and

meat by-products of beef cattle, sheep, and hogs, and a 5 ppm feed additive tolerance to cover use of cyromazine in poultry feed (see review by A. Rathman, PP#9G2230/FAP# 2H5338). Presently, there are no permanent tolerances established.

Conclusions

1. The residues of concern in poultry tissue and eggs are the parent compound, cyromazine, and its metabolite, melamine.

2. Adequate methodology is available for gathering residue data. The method's adequacy for enforcement purposes will be determined by the method tryout which is pending. The petitioner does need to submit analytical methodology without "Confidential" being stamped on it.

3. The petitioner should submit data showing whether or not nitroso-cyromazine is present in the technical product.

4a. The tolerance request of 0.4 ppm in poultry meat, fat, and meat by-products, is adequate for this request proposed use.

4b. The requested feed additive tolerance of 5 ppm is adequate. The tolerance expression should be proposed as indicated under Recommendations.

5. An international residue limit status sheet is included. There are no internationally established tolerances for this chemical.

6. To avoid illegal residues in crops from this use, EFB has concluded that the application of poultry manure (containing cyromazine) to crop land be limited to 5 tons/acre. We concur with this statement.

Recommendation

We recommend against the proposed tolerance until the questions raised in conclusions 2, 3, 4(b) and 6 are resolved.

A feed additive regulation should be proposed as follows:

The additive cyromazine (N-cyclopropyl-1,3,5-triazine-2,4,6-triamine) may be safely used in accordance with the following prescribed conditions:

a. It is used as a feed additive in the feed of poultry at the rate of 0.003-0.01 pound per ton of poultry feed.

2

b. It is used for control of certain flies in manure of treated poultry.

c. To assure safe use of the additive, the label and labeling of the pesticide formulation containing the feed additive shall conform to the label and labeling registered by the U.S. Environmental Protection Agency.

Detailed Considerations

Manufacturing Process

The manufacturing process and the composition of the technical material were adequately discussed in our review of PP#9G2230 (See our review by A. Rathman, November 14, 1979).

[redacted]

Technical CGA-72662 is a minimum of 95% pure by weight. The low level of impurities the total of which will not exceed 5% of the technical product, are not expected to cause a residue problem.

For any permanent tolerance submissions, the petitioner was informed in our review of February 19, 1980 (A. Rathman, PP#9G2230) of the need for data showing whether or not nitroso-CGA-72662 is present in the technical product. No information has been submitted to address this question. This deficiency remains outstanding.

Formulation

This product will be formulated as Larvadex 0.3% Premix containing 0.3% a.i. and 99.7% inerts [redacted]

For previous temporary tolerance requests, this pesticide was also formulated as 5% SCO (soluble concentrate) containing 5.3% technical material and 94.7% [redacted] We had deferred to TOX as to their concern for [redacted] in poultry feeds. The petitioner does not appear to be considering this formulation, therefore the deferral to TOX for [redacted] in poultry feed is moot for this permanent tolerance request. If in the future the petitioner wishes to use this SCO formulation, we will defer to TOX as to their concern for [redacted] as a direct feed additive.

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

INERT INGREDIENT INFORMATION IS NOT INCLUDED

Proposed Use

To control the housefly and soldier fly mix cyromazine in poultry feed at the rate of 0.003 lb. a.i./ton of feed. To control the lesser housefly mix 0.01 lb. a.i., cyromazine per ton of poultry feed. Treated feed is used as a daily ration for as long as particular pest is a problem. There is a restriction against feeding treated feed to broiler poultry or poultry producing eggs for hatching purposes.

Nature of the Residue

Because this is an animal feed-through use, only animal metabolism studies are being considered. Rat (Report No. ABR-78072), chicken (Report Nos. ABR-79043 and ABR-81035), goat (Report Nos. ABR-80051 and Biol-80006, and sheep (Report No. ABR-79056) metabolism studies have been submitted. Report Nos. ABR-78072 (rat), ABR-79043 (poultry) and ABR-79056 (sheep) have previously been adequately reviewed (see memo by A. Rathman, PP#9G2230, November 14, 1979) and will only be included in the summary. The newly submitted metabolism studies in poultry and goats will be discussed in turn.

Poultry-Report Nos. Biol.-81002 and ABR-81035

Chickens were orally dosed in feed for 7 consecutive days with universally ring labeled ^{14}C-Cyromazine at levels of 7.7 ppm, 32.9 ppm and 84.3 ppm (2 chickens per treatment). These dosages represent approximately 1X, 6X and 16X the proposed rate of 5 ppm fly larvicide in poultry feed. Chickens were sacrificed 24 hours after the last dose. Daily egg samples were taken and total radioactivity determined in separated yolk and white fractions. Residues in all samples plateaued by the 4th to 6th day. The amount of total activity (calculated as parent compound) in both yolks and whites continued to increase with increasing treatment. At the 1X, 6X and 16X feeding levels, maximum radiolabel (calculated as cyromazine) was reported as 0.23 ppm, 1.26 ppm, and 3.21 ppm in whites, and 0.17 ppm, 0.85 ppm and 2.29 ppm in yolks, respectively. Only liver tissue was analyzed for radiolabeled metabolites. This organ showed a dose response effect with sample radioactivity increasing as feed levels increased; 0.03 ppm, 0.19 ppm and 0.47 ppm equivalent cyromazine in liver reported for 1X, 6X and 16X feeding levels (See Poultry Report ABR-79043 for radioanalysis of other chicken tissue). Six day egg yolk and white samples and liver at the 1X and 16X dose level, were extracted with methanol and water. After clean-up, extracts were analyzed for cyromazine and melamine using GC/MS analysis. Of the total activity in all samples, 82 to 93% were extractable.

In egg white and yolk, cyromazine was the major residue (up to 86.3% of extractable activity). Melamine varied from 1 to 3.8% of the extractable activity; however, one egg white sample (1X treatment level) had a reported melamine and cyromazine concentration of 38.3% and 18.4%, respectively. In this same sample a metabolite A, not reported before and surmised to be ammeline (see table of structures below), was determined as 19.9%. The petitioner attributed these results to biological variability of individual animals occasionally encountered.

Analysis of liver sample indicated the major residue is the parent compound (62-84% of the extractable activity), while melamine was the major identified metabolite (6-7%) at both feeding levels (1X and 16X).

Goat Study (Report Nos. Biol-80006, November 11, 1980 and ABR-80051, December 29, 1980)

A lactating goat was treated for 10 consecutive days with capsules of [U-ring-^{14}C]-cyromazine at the rate of 5 ppm in the feed. Total recovery of the administered dose was 88.7%. The bulk of the label was discharged through the urine (83.1%); the remaining label was found in feces (4.4%), blood (0.03%), tissues (0.26%), milk (0.10%), and gastrointestinal tract (0.85%). No radionuclide was reported associated with expired CO_2 or volatiles, implying that the triazine ring may remain intact.

Concentrations of equivalent ^{14}C-Cyromazine in various tissues were reported as 0.25 ppm in liver, 0.16 ppm in the gastrointestinal tract, 0.024 ppm in kidney and 0.004 ppm or less in all other tissues. Milk had a maximum level of radioactivity equivalent to 0.02 ppm. The level of activity in milk appeared to remain relatively constant over the 10-day experimental period (0.012-0.019 ppm). Only a limited identification of radioactivity was reported for urine, feces, liver, and milk. Both thin layer chromatography/radioautography and ion exchange chromatography were used to characterize the samples. Using TLC, 65% of the labeled material in urine co-chromatographed with standard cyromazine, while 6% of a minimum of two unknown metabolites and 26% of a major unidentified metabolite were also reported. Sixty-eight percent of the activity in goat feces was extractable. Of this amount, 44% was characterized as cyromazine, while unidentified radioactivity, which may represent two additional metabolites, was reported as 12% and 13% of extractable label. When day 10 milk containing 0.02 ppm equivalent cyromazine was fractionated, 87.3% of the radioactivity was found in the whey and lesser amounts in casein (10.3%) and fat

(2.4%). The only residue identified in whey was cyromazine (15% of ^{14}C in whole milk). Eighty percent of the liver radiolabel was extractable. Using TLC, only 1% of this nuclide was cyromazine. The major unidentified metabolite was reported as 75% of label in liver.

Summary

Metabolism studies of ^{14}C-Cyromazine in rats, chickens, sheep and goats have been submitted. In rats, goats and sheep most of the administered activity use excreted in the urine (83-95%), with a few percent excreted in feces. At the 5 ppm feeding level, little activity (calculated as cyromazine) was detected in rat tissue (some detectible radiolabel in liver <0.007 ppm), while in goats and sheep small amounts of activity (calculated as cyromazine) were detected in liver (0.17-0.25 ppm), kidney (0.024-0.048 ppm) gastrointestinal tract (0.16 ppm) and other tissues (0.013 ppm or less).

Unmetabolized cyromazine accounted for the major radioactivity in feces (44%) of goat and sheep only, and urine (65-84%) of rats and sheep. In sheep liver cyromazine accounted for 12% of the total administered activity, while 43% of the activity was determined to be melamine. Much of the remaining radioactivity in sheep feces and liver were "nonextractables" (42-44%). In goat liver, only 1% of the radionuclide was identified as parent compound. Of the remaining portion of unidentified metabolites, one compound was reported as 75% of total in liver.

In poultry the amount of total radioactivity (calculated as parent compound) appearing in egg white and yolk is reported as 0.09-0.22 ppm and 0.08-0.15 ppm, respectively, at the 5 ppm (1x) feeding level. These values increase with increasing feeding dose of ^{14}C-cyromazine. At the 84.3 ppm (16x) feeding level egg white and yolk have maximum reported radiolabel of 3.2 ppm and 2.29 ppm equivalent cyromazine. Poultry skin, fat, muscle, heart, gizzard, kidney, liver and by-products were subjected to radioanalysis. Radioactivity in all tissue ranged from <0.002 to 0.037 ppm at the 5 ppm (1x) feeding level. Liver, representing a 16x dose level had a reported equivalent cyromazine residue of 0.47 ppm. In egg white and yolk, cyromazine was the major residue reported as up to 86.3% of the extractable activity. Melamine varied from 1 to 3.8% of the extractable activity. Liver, containing the highest residue, was the only tissue extracted and its labeled residue identified. The major residue in liver was the parent compound (62-84% of the extractable activity) and melamine was the major identified metabolite (6-7%).

6

The metabolism of cyromazine in poultry is adequately delineated for this permanent tolerance request. The residue of concern in eggs and poultry tissue is the parent compound, cyromazine, and its metabolite, melamine.

Analytical Method

Analytical methods have been submitted for cyromazine and its major metabolite, melamine. Method No. AG-341 determines cyromazine while Method No. AG-364 determines both cyromazine and its metabolite, melamine. For Method No. AG-341, the petitioner reports a level of sensitivity of 0.1 ppm for eggs, 0.1 ppm for tissue, and 1.0 ppm for feed samples. For Method No. AG-364, the level of sensitivity is reported as 0.05 ppm in eggs and 0.1 ppm in tissue for cyromazine, and 0.04 ppm for melamine (in both eggs and tissues), equivalent to 0.05 ppm of cyromazine. Method No. 341 was described in PP #9G2230 (A. Rathman, November 14, 1979). In brief, sample (poultry eggs and tissue) is extracted with methanol, cleaned up by successive chromatography on Dowex 50W, X-4 cation exchange resin, silica gel column and liquid-liquid column. Chicken feed samples are extracted with glacial acetic acid, the extractant evaporated to dryness and cleaned up using silica gel column chromatography. Qualitation and quantitation is completed using gas chromatography with an alkali flame ionization detector in the nitrogen-specific mode. At fortification levels of 0.1 to 0.5 ppm, recoveries are reported as 68-73 $\pm$ 13%; in feed validated at 2.5 to 25 ppm, recovery is reported as 89 $\pm$ 13%.

Using Method No. AG-364, which determines both parent and its metabolite (melamine), sample is blended with dry ice, extracted with 90% methanol/water and filtered. The extract is initially cleaned up using a Dowex 50W-X4 cation exchange column in methanol. The sample, adsorbed to column, is washed with methanol. Elution of the column is accomplished with a concentrated ammonium hydroxide:methanol solution at 1:3. The sample is evaporated to dryness. To the dried sample is added 1 ml of McIlvaine's buffer, pH 7 (82.4 ml of 0.2 M disodium phosphate and 17.6 ml of 0.1 M citric acid). Final clean up is then accomplished using a Hy-Flo Super-Cel column. The eluate is again dried down and redissolved in methanol and quantitated using GC/MS. Cyromazine is determined using single ion monitoring at m/e = 167 $\pm$ 0.2 amu, and after reinjection into the GC/MS, melamine is determined by single ion monitoring at m/e = 127 $\pm$ 0.2 amu. Recovery of cyromazine, at fortification levels of 0.05-0.2 ppm, in eggs and tissues is reported as 78 $\pm$ 8% and 84 $\pm$ 18%, respectively; while for melamine in eggs and tissues at fortification levels of 0.040.4 ppm, recovery is reported as 84 $\pm$ 10% and 77 $\pm$ 15%, respectively.

Studies were submitted to indicate the specificity of the analytical methodology. For Method AG-341, 54 of 61 pesticide chemicals presently having established tolerances for poultry meat, fat, and eggs were used in this study. Specificity validation was performed at the established tolerance level of the respective pesticides. No interferences were reported. The seven pesticides unavailable for testing have established tolerances of 0.1 ppm or less.

The specificity of Method AG-364 was determined using 68 of 71 pesticides having permanent or section 18 tolerances in/on poultry meat, fat, and eggs. None of these compounds exhibited interferences using this methodology. The high specificity of GC/MS using single-ion monitoring makes interferences of the remaining untested pesticides unlikely.

Finally, freezer storage (5°F) studies were submitted to determine the stability of cyromazine treated samples. Using Methods AG-341 and AG-364, sample of poultry eggs were fortified at 0.2 ppm, stored at 5°F for up to 23 months and analyzed. Recoveries ranged from 52 to 94%.

We are awaiting the results of a method-try-out request before commenting on the adequacy of the above methods for enforcement purposes.

Residue Data

Mature Leghorn layers were fed cyromazine in their feed at levels of 0, 2.5, 5.0 and 25.0 ppm for 27 days. Samples of breast, fat, liver, skin and thigh muscle were taken 14 and 27 days after start of treatment and analyzed using Method AG-341. Residues of cyromazine in fat, breast, thigh and skin were reported as <0.05 ppm for the 14 and 27 day sample periods at the 2.5 ppm feeding levels. Liver at the same feeding level was 0.08 ppm and <0.05 ppm at day 14 and day 27, respectively. At the 5 ppm feeding level, residues of cyromazine in fat, liver, breast, thigh and skin were reported as <0.05 ppm, 0.09-0.10 ppm, <0.05-0.07 ppm, 0.06 ppm and <0.05 ppm, respectively, at day 14 and day 27. For the 25 ppm feeding level, fat, liver, breast, thigh and skin had reported residues of <0.05 ppm, 0.41 ppm, 0.19-0.28 ppm, 0.39-0.55 ppm, and 0.09-0.15 ppm, respectively. Controls were <0.05 ppm.

A second residue study was conducted and determined both cyromazine and its metabolite, melamine, in poultry tissue and eggs using Method AG-364 (Method AG-341 for parent in eggs only). Treatment levels were 5, 25, and 50 ppm for 28 days. Samples of eggs were taken every couple of days.

At day 0 and day 1 eggs contained <0.05 ppm parent compound at all feeding levels. For day 3 to day 28 samples, residues of parent compound were reported as 0.08-0.16 ppm, 0.32-0.80 ppm, and 1.1-2.4 ppm for the 5 ppm, 25 ppm, and 50 ppm feeding levels, respectively. Residues of the metabolite, melamine, for day 1 samples were <0.05 ppm for all feeding levels. Day 3 to day 28 samples had reported melamine residues of <0.05-0.17 ppm, <0.05-0.25 ppm and 0.07-0.22 ppm. Poultry tissue was examined at 7, 14, 21, and 28 days. The following results were reported:

Feed Level (ppm)	Interval (days)	Cyromazine (ppm)			
		Fat	Skin	Meat	Liver
5	7-28	<0.05	<0.05-0.06	0.05-0.08	0.08-0.13
25	7-28	<0.05	<0.11-0.30	0.24-0.43	0.30-0.75
50	7-28	<0.05	<0.13-0.40	0.57-1.1	0.63-1.1
5	7-28	<0.05*(0.2)	<0.05-0.13	<0.05-0.22	<0.05-0.09
25	7-28	<0.05	<0.05-0.12	<0.05-0.14	0.05-0.12
50	7-28	<0.05-0.07	<0.05-0.07	<0.05-0.10	0.07-0.14

To check proper mixing and stability, feeds mixed with 2.5 ppm, 5.0 ppm, 25 ppm, and 50 ppm of cyromazine were analyzed on day 0 and day 7. For the day 0 samples, analyzes indicated 2.0-2.8 ppm, 3.5-6.4 ppm, 6.8-32 ppm, and 43-70 ppm cyromazine for feeds mixed at the 2.5 ppm, 5 ppm, 25 ppm, and 50 ppm levels, respectively. For the day 7 samples, analyses indicated 1.7-2.6 ppm, 3.9-5.6 ppm, 18-29 ppm and 55 ppm cyromazine for mixtures at the 2.5 ppm, 5 ppm, 25 ppm and 50 ppm levels, respectively. From the above studies, cyromazine appears stable when mixed in weekly batches of poultry feed.

From the above data the requested tolerances of 0.4 ppm in poultry eggs, meat, fat and meat by-products, and 5 ppm in poultry feed are adequate for these proposed uses. When the tolerance is finalized it should be written as discussed under our "Recommendations" above.

Other Considerations

Because these tolerance requests pertain to use in poultry feed only. There is no concern for residues of cyromazine in other livestock meat and milk.

9

Chicken manure containing cyromazine can be applied to pastures and cropland. Residues for this inadvertent use of cyromazine in pasture grass and cropland is adequately discussed in the Environmental Fate Branch review of Sam Creeger (Registration/File No. 100-AGR and 100-AGE, Cyromazine, 10/1/82). To avoid illegal residues in crops, EFB has concluded that application of poultry manure, containing residues of cyromazine, be restricted to 5 tons/acre of cropland.

INTERNATIONAL RESIDUE LIMIT STATUS

HEMICAL Cryomazine PETITION NO 2F2707/2H5355

CCPR NO. None

Codex Status

/ / No Codex Proposal
/ / Step 6 or above

Residue (if Step 9): _____

Crop(s)	Limit (mg/kg)

Proposed U.S. Tolerances

For 180. new (40 CFR)
and
561. new (21 CFR)

Residue:* Cryomazine and metabolite,** melamine

Crop(s)	Tol. (ppm)
eggs	0.4
poultry (m, f, mbp)	0.4
poultry feed	5.0 (FA)

CANADIAN LIMIT

Residue: _____

Crop	Limit (ppm)
none	

MEXICAN TOLERANCIA

Residue: _____

Crop	Tolerancia (ppm)
none	

Notes: *Cryomazine (N-cyclopropyl-1,3,5-triazine-2,4,6-triamine) and its principal metabolite, melamine (1,3,5-triazine-2,4,6-triamine).

**Metabolite not included in proposed FA tolerance.

cc. R.F., S.F., PP#2F2707/FAP#2H5955, TOX, Thompson, FDA, USDA, Errico

DCR-26267:Philip Errico:RCB-13:Rm810:47377:2/10/83:efs
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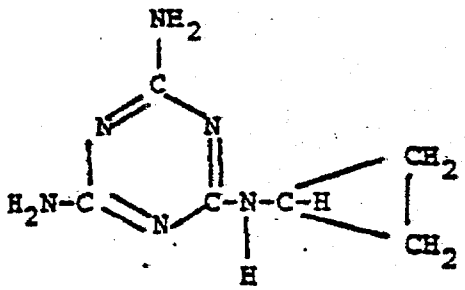
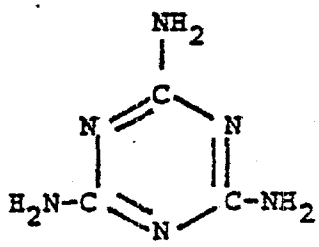
REVISED-2/22/83:DCR-26381:P.Errico:efs

12



CGA-72662

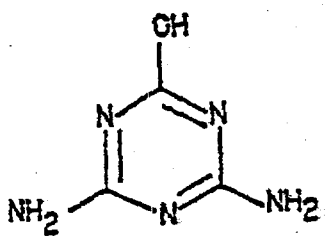
N-cyclopropyl-1,3,5-triazine-2,4,6-triamine



Melamine

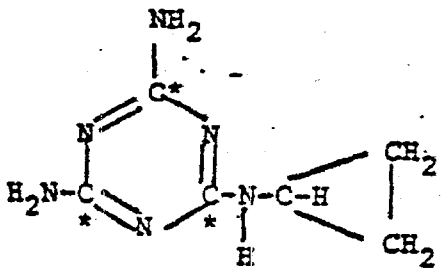
1,3,5-triazine-2,4,6-triamine

Figure 1. Code Numbers, Structures, and Nomenclature for CGA-72662



GS-17791, ammeline

$C_3H_5N_5O$



Δ^{14}C-CGA-72662

[U-ring-^{14}C]-N-cyclopropyl-1,3,5-triazine-2,4,6-triamine

Figure 1. Code Numbers, Structures, and Nomenclature for CGA-72662 (Continued)